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Seroprevalence of *Neospora caninum* in non-carnivorous wildlife from Spain

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Abstract

Serum samples from 1034 non-carnivorous wildlife from Spain were tested for antibodies to *Neospora caninum* by competitive screening enzyme linked immunosorbent assay (ELISA) and confirmed by an indirect fluorescent antibody test (IFAT). High agreement was observed between results in both techniques (kappa value higher than 0.9). Prevalences of *N. caninum* antibodies positive by both techniques were 11.8% of 237 red deer (*Cervus elaphus*), 7.7% of 13 barbary sheep (*Ammotragus lervia*), 6.1% of 33 roe deer (*Capreolus capreolus*) and 0.3% of 298 wild boar (*Sus scrofa*). In one of 53 hares (*Lepus granatensis*), antibodies were found in the ELISA but could not be confirmed by IFAT due to lack of sample. Antibodies to *N. caninum* were not found in any of 251 wild rabbits (*Oryctolagus cuniculus*), 79 fallow deer (*Dama dama*), 27 mouflon (*Ovis ammon*), 40 chamois (*Rupicapra pyrenaica*) and three Spanish ibex (*Capra pyrenaica*). Statistically significant differences were observed between *N. caninum* seroprevalence in red deer and management of hunting estates (open versus fenced) with higher prevalence in fenced estates, and among sampling sites. Seroprevalence was particularly high in some areas (MO estate in South-Central Spain or some estates of Catalonia, North-East Spain), while no contact with *N. caninum* was observed in others. Results indicate that in certain areas of Spain, *N. caninum* is present in wildlife, especially in red deer. These results have important implications in both sylvatic cycles and may influence the prevalence of infection in cattle farms in those areas. To our knowledge, this is the first report of antibodies to *N. caninum* in wildlife from Spain and the first report of *N. caninum* antibodies in barbary sheep and wild boar.

Keywords: *Neospora caninum*; Wildlife; Spain; ELISA; IFAT

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1. Introduction

Neospora caninum is one of the most important causes of abortion in cattle worldwide (reviewed by Dubey and Lindsay, 1996; Dubey, 2003). It can also cause mortality in other livestock species, companion animals, and in wildlife species. Antibodies to *N. caninum* have been reported in a number of free-ranging and captive wild animals such as black and white tailed deer, water buffaloes, African buffaloes, elands, Thompson gazelles, impalas, red deer, chamois, roe deer, alpine ibex, warthogs, zebras, camels, musk ox, rhinoceros (reviewed by Dubey, 2003; Ferroglio et al., 2003; Soldati et al., 2004), moose (Gondim et al., 2004b), American bison and caribou (Dubey and Thulliez, 2005), *Mazama* spp. (Tiemann et al., 2005a), pampas-deer (Tiemann et al., 2005b) and from zoo species such as blackbuck, European bison, lechwe, sitatunga, Thorold's deer, Eastern elk, Vietnam sika deer and Pere David's deer (Sedlák and Bártová, 2006), suggesting a widespread exposure to *N. caninum* in these species.

Many aspects of the life cycle of *N. caninum* are unknown. The dog and the coyote (*Canis latrans*) are the only definitive hosts known that can excrete the environmentally resistant oocysts (Gondim et al., 2004a; McAllister et al., 1998). The role of wildlife in the life cycle of *N. caninum* is uncertain. In North America the white-tailed deer is considered to be its main reservoir host and viable *N. caninum* has been isolated from this host (Gondim et al., 2004b; Vianna et al., 2005).

In Europe, few seroprevalence studies of *Neospora* infection in wild ruminants have been performed (Ferroglio and Rossi, 2001; Sedlák and Bártová, 2006). Red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) are the most popular and abundant big game species in Spain (Gortazar et al., 2000; Tellería and Saez-Royuela, 1986), while wild rabbits are the main small game species (Ministry of Agriculture, Fisheries and Food, 1996). We analyzed the presence of antibodies against *N. caninum* in 1034 wild (Table 1) to provide information on the epidemiology of *N. caninum* infection in non-carnivorous animals from Spain.

2. Materials and methods

2.1. Source of animals

The serum samples were obtained from animals shot or captured during the hunting season from 1993 to 2005. All sera had been collected previously for use in other investigations. Samples from Central and South Spain were generously provided by IREC (CSIC-UCLM-JCCM), Ciudad Real and samples from Catalonia were generously provided by Wildlife Ecopathology Service, Veterinary School, Autonomous University of Barcelona.

Red deer were classified into three age groups, young red deer ($\leq$1 year old), juveniles (between 1 and 4 years old), and adults ($\geq$4 years old). Management of hunting estates was classified as open (open hunting areas) or fenced (fenced hunting areas). The red deer samples from Catalonia, provided by Wildlife Ecopathology Service of Autonomous University of Barcelona were collected between 1993 and 2005, while the rest of samples from other areas of Spain provided by IREC were collected from 2001 to 2003.

2.2. Sampling sites

Red deer samples were collected in different estates from Northeast Spain (Catalonia), from South Spain

Table 1
Prevalence of *Neospora caninum* antibodies in 1034 wildlife from Spain

Species	No. examined	No. positive[c]	Prevalence (%) ± 95% CI
Red deer (*Cervus elaphus*)[a]	237	28	11.8 ± 4.1
Barbary sheep (*Ammotragus lervia*)[a,b]	13	1	7.7 ± 14.5
Roe deer (*Capreolus capreolus*)[a,b]	33	2	6.1 ± 8.2
Wild boar (*Sus Scrofa*)[b]	298	1	0.3 ± 0.6
Spanish ibex (*Capra pyrenaica hispanica*)[b]	3	0	0.0 ± 0.0
Wild rabbit (*Oryctolagus cuniculus*)[b]	251	0	0.0 ± 0.0
Hare (*Lepus granatensis*)[b]	53	1[d]	0.0 ± 0.0
Fallow deer (*Dama dama*)[b]	79	0	0.0 ± 0.0
Mouflon (*Ovis ammon*)[b]	27	0	0.0 ± 0.0
Chamois (*Rupicapra pyrenaica*)[b]	40	0	0.0 ± 0.0

[a,b] Different letters indicate values of prevalence significantly different between species in column ($p < 0.05$).
[c] Screeing ELISA and confirmatory IFAT $\geq$1:50.
[d] Not confirmed by IFAT.

(Andalucia: Sierra Morena) and from Central Spain (Castilla-La Mancha: Guadiana) (Table 2).

Wild boar samples were collected from areas in Northern Spain (Asturias, Castilla y León and Catalonia), from South Spain (Andalucía: Sierra Sur de Jaén and Sierra Morena) and from Central Spain (Castilla-La Mancha: Guadalajara, Montes de Toledo and Guadiana).

Wild rabbit samples were collected from areas in Northern Spain (Aragón and Catalonia), from South Spain: (Andalucía: Huelva and Cádiz) and from Central Spain (Castilla-La Mancha: Toledo). Hares samples were collected in Central Spain (Madrid and Castilla-La Mancha: Toledo, Ciudad Real and Albacete) and from South Spain (Andalucia: Seville).

Fallow deer, roe deer, wild goat, chamois, mouflon and barbary sheep samples were surveyed from different areas in Central and South Spain. No samples from these species were available from Catalonia (Further information can be found in Gauss et al. (2006)).

Table 2
Prevalence of *N. caninum* antibodies in 237 red deer from Spain by hunting estates

Areas of hunted deer	Hunting estates	No. examined	No. positive[a]	Prevalence (%) ± 95% CI
North-East Spain (Catalonia)		82[b]	8	9.7 ± 6.4
	Vallcalent	4	3	75.0 ± 42.4
	Vic	4	3	75.0 ± 42.4
	Bellver	10	2	20.0 ± 24.8
	Andorra	13	0	0.0 ± 0.0
	Berga	7	0	0.0 ± 0.0
	Butsenit	4	0	0.0 ± 0.0
	Boumort	18	0	0.0 ± 0.0
	Terrades	6	0	0.0 ± 0.0
	Vall d'Aran	10	0	0.0 ± 0.0
South-Central Spain (Guadiana)		89	19	21.3 ± 8.5
	CT	1	0	0.0 ± 0.0
	MO	88	19	21.6 ± 8.6
South Spain (Sierra Morena)		66	1	1.5 ± 2.9
	RA	20	1	5.0 ± 9.5
	SE	21	0	0.0 ± 0.0
	LN	25	0	0.0 ± 0.0

[a] Screening ELISA and confirmatory IFAT ≥1:50.
[b] There was not available data from six animals concerning to sample site.

2.3. Serological examination

Blood samples collected from the heart and/or thorax region of all game species during necropsy were centrifuged and sera obtained were stored at −20 °C until assayed for antibodies to *N. caninum*.

A commercial ELISA *N. caninum* monocupule screening kit from laboratories Pourquier (P00510/02) (France) was used for detection of *N. caninum* antibodies in wildlife according to the manufacturers' instructions. Basically, 50 μl samples were diluted (1:2) and incubated in the wells coated with specific *N. caninum* antigen. After washing, a monoclonal anti-*N. caninum* antibody coupled to peroxidase was added and samples incubated. After washing, substrate (3,3′,5,5′-tetramethylbenzidine, TMB) was added to the plates. The reaction was stopped by addition of sulphuric acid 0.5 M and the plates were read at 450 nm absorbance. In addition to the positive and negative control samples provided in the kit, *Neospora* highly positive samples and negative samples from bovine and dogs previously analyzed in our lab by other validated commercial ELISA and IFAT techniques were included.

Confirmation of samples found positive by ELISA was sought by an indirect immunofluorescence test (IFAT). Individual sera were tested for presence of IgG antibodies to *N. caninum* by IFAT as described previously by Ortuño et al. (2002).

Sera from all the seropositive animals observed in the ELISA (35 with available sera) were analyzed together with 57 randomly selected negative sera from different species, 21 from negative red deer, in a blind assay performed by a single person and only animals that were positive to both techniques were considered positive.

All sera samples were also tested for antibodies to *Toxoplasma gondii* by the modified agglutination test (MAT) (Dubey and Desmonts, 1987) to find potential cross-reactivity between these two parasites.

2.4. Statistical analysis

Levels of agreement between serological test (competition ELISA and IFAT) were performed using the kappa statistics.

Seroprevalence was statistically analyzed considering the variables species, geographical area, estate management (open versus fenced), year of sample collection, sex and age of the animals.

The statistical data analysis was performed using the SPSS 12.0 Statistical Program by chi-square test. The differences among more than two variables were

analyzed by one-way ANOVA. The differences between variables were analyzed by Bonferroni or Tukey–Kramer multiple comparison tests. When variances were not homogenous, Kruskal–Wallis non-parametric ANOVA and Dunn's test for multiple comparisons were performed (species, geographical area, and year of sample collection variables). The differences were considered statistically significant when $p \leq 0.05$. The confidence intervals (95% of confidence level) of seroprevalence of *N. caninum* antibodies were calculated based on Martin et al. (1987).

3. Results

Antibodies to *N. caninum* were detected in 36 of 1034 wildlife samples by ELISA (30 from red deer, two from wild boars, two from roe deer, one from barbary sheep, and one from a hare). In the latter case, there was not enough serum to confirm the results by IFAT. Of the 35 remaining positive samples in the screening ELISA, 32 were also found positive by IFAT (28 samples from red deer, one from wild boar, two from roe deer and one from barbary sheep), with a kappa value of agreement between both serological tests of 0.929. When only data from red deer were included in the agreement analysis, the kappa value was 0.920. Antibody titers of the 28 positive red deer samples confirmed by IFAT were 1:50 in five, 1:200 in two, 1:400 in seven, 1:800 in seven and 1:1600 in seven animals. IFAT titers of other animals were: 1:50 in one wild boar, 1:200 in one barbary sheep and 1:100 in two roe deer.

The prevalence of antibodies to *N. caninum* in samples positive to both tests were 11.8% in red deer, 6.1% roe deer, 7.7% barbary sheep, and 0.3% wild boar (Table 1).

There were statistically significant differences in prevalence among species ($p = 0.001$), with significantly higher seroprevalence in red deer compared to wild boars, wild rabbits, hares, fallow deer, chamois, mouflon and Spanish ibex but not between red deer, roe deer and barbary sheep (Table 1).

When *N. caninum* seroprevalence data in red deer were examined in more detail, statistically significant differences ($p = 0.001$) were observed in the prevalence of infection among sampling areas (Table 2). The highest prevalence of infection was observed in Guadiana (21.3% of 89 samples) in Central-South Spain. In this area all 19 positive red deer were found in one estate (MO) (Table 2). Interestingly, the positive barbary sheep observed in the present study, together with one wild boar considered positive in the ELISA test but not confirmed by IFAT, were also from the same estate. The second area of higher prevalence of infection in red deer was Catalonia (9.8% of 82 samples) in North-East Spain. In this area, statistically significant differences ($p = 0.006$) were observed among hunting estates. Out of nine hunting estates sampled, positive animals were observed in three of them with high prevalence of infection (Vallcalent 75% of four samples), Vic (75% of four samples) and Bellver (20% of 20 samples) (Table 2). In South Spain, in the Sierra Morena area (SM) only one positive red deer of 66 samples (1.5%) was found from RA estate (Table 2).

Statistically significant differences ($p = 0.036$) were observed between seroprevalence in red deer infection and management of estates (open versus fenced). Antibodies were only found in red deer from fenced areas (28 positive of 203 animals), with no positive samples of 28 animals from open areas. No management data were available from six animals.

Statistically significant differences in antibodies prevalence of *N. caninum* and sex or age were not observed. Antibodies were found in 11 of 115 males and in 17 of 121 females with sex data not recorded from one deer ($p = 0.529$); and in three of 31 young, in four of 37 juveniles and in 21 of 166 adults with no age data available from three animals ($p = 0.872$).

Statistically significant differences were observed in the prevalence of *N. caninum* infection among years of collection of samples ($p = 0.001$), although this fact was related to different hunting estates that were sampled every year (data not shown). In the Catalonian hunting estates, the dynamics of antibodies could not be determined because number of samples in each estate was limited and most of the areas were sampled only 1 year. The only estate sampled three consecutive years was MO estate in Guadiana. Prevalence of infection in this estate was 16.7% of 12 samples in 2001, 7.3% of 41 samples in 2002 and 40% of 35 samples in 2003 with significant higher prevalence of infection observed in 2003 ($p = 0.002$). It was interesting to find out that the only times that hunting with dogs had taken place in the estate were in 2002 and 2003 and there had not been any more hunts with dogs since then. Another interesting data was that in 2001, five new deer entered in this, otherwise closed, estate.

We decided to further investigate this estate (MO) to clarify the role of dogs and presence of infection in earlier years. Two sera were available from the red deer introduced in 2001. Thirty-seven additional samples from retrospective samplings in deer from years 2000 (20 samples) and 2001 (17 samples) were analyzed and 15 dogs present in the estate were bled to know their serological status. At the time of the new sera analysis,

Institut Pourquier had changed the original kit for a new version (P00511/01) that used an anti-cattle IgG conjugate instead of the anti-*N. caninum* conjugate, therefore, red deer samples were analyzed by this new screening test and confirmed by IFAT, while dog samples were analyzed directly by IFAT. Prevalence of infection in the additional red deer samples was 35% (seven positive samples with titers 1:200 in one, 1:400 in two, 1:800 in two and 1:1600 in two samples) in 2000 and 11.8% (two positive samples with titers 1:1600 and 1:3200, respectively) in 2001. Antibodies were not found in any of the two deer introduced in 2001 and in any of the dogs present in the estate.

All samples used in this survey were tested for both *N. caninum* and *T. gondii*. In 32 samples that tested positive for *N. caninum* antibodies, 23 (71.9% of the positive *N. caninum* samples) reacted solely to *N. caninum*. Of the 28 red deer samples positive to *N. caninum*, 19 (67.8%) samples reacted solely to *N. caninum*. In particular, in the MO estate 16 sera reacted solely to *N. caninum*, seven sera reacted solely to *T. gondii* and only three sera reacted to both parasites.

Antibodies to *T. gondii* (MAT >1:25) were found in 21.1% of red deer. Seroprevalence against *N. caninum* was found to be higher than that for *T. gondii* in MO estate in Guadiana and Vic and Vallcalent in Catalonia (data not shown).

4. Discussion

Antibodies against *N. caninum* were analyzed by screening ELISA and confirmatory IFAT. The commercial ELISA used in this study has been validated for bovine sera. Its principle of competition makes this test theoretically possible to be used in any other species but validation data are not yet available for these species. Therefore, we confirmed positive samples observed in the ELISA test by IFAT. The IFAT is a well-established technique for detecting anti-*N. caninum* antibodies in different animal species. Most of the surveys of seroprevalence of *N. caninum* in different species, such as dogs, have been based on IFAT using 1:50 as a cut-off value (Cheadle et al., 1999; Ortuño et al., 2002) and, thus we also used a cut-off at 1:50. Antibodies to other protozoans such as *T. gondii*, *Sarcocystis* spp. and *Babesia canis* do not cross-react with *N. caninum* tachyzoites in the IFAT at dilutions of 1:50 or higher (Dubey and Lindsay, 1993). Wolf et al. (2005) using the immunoblot as reference technique found that IFAT exhibited a sensitivity and specificity of about 95% analysing *N. caninum* antibodies in sera from South American camelids. In the present study, a high level of agreement in the results with the ELISA was observed (kappa value higher than 0.90). In addition, more than 70% of the positive samples reacted solely to *N. caninum* and not to *T. gondii*, indicating less of cross-reaction between these two closely related protozoans.

Some researchers have used higher titers in the IFAT as cut-off when analysing wildlife. Gondim et al. (2004b) used cut-off in IFAT at ≥1:100 for deer and moose sera and 1:50 was selected as the cut-off for coyote and wolf sera. Chávez-Velásquez et al. (2004) observed that in llamas and alpacas IFAT titers equal to or higher than 1:100 proved to be truly positive when confirmed by Western blot while titer equal to 1:50 could be due to cross-reactions with other closely related apicomplexan parasites and could lead to an overestimation of the prevalence of infection. Even in the case of using a different cut-off in the present study, our results would remain similar, since in the present study IFAT titers of 1:50 were only observed in five red deer sera of 28 positive animals, with titers over 1:100 in 23 animals. IFAT titers in barbary sheep and roe deer were also higher than 1:100. Besides, no correlation was observed with the presence of *T. gondii* antibodies.

To our knowledge, this is the first report of presence of *N. caninum* antibodies in wildlife from Spain and the first report of *N. caninum* antibodies in wild boar and in barbary sheep, an introduced non-cervid ruminant species member of the Bovidae Family.

The presence of antibodies to *N. caninum* in a wild boar from Spain was confirmed by IFAT. This may indicate the first natural *N. caninum* infection in wild boars. Wild boars are omnivorous animals that root the ground to search for food and they consume a large variety of plant material, live and dead animals (Schley and Roper, 2003). In domestic pigs, experimental infection (Helmick et al., 2002; Jensen et al., 1998) and more recently natural infection (Damriyasa et al., 2004) have shown a low incidence of *N. caninum* infection, suggesting that environmental exposure to *N. caninum* occurs rarely in domestic pigs (Damriyasa et al., 2004; Helmick et al., 2002). This also seems to be the case in wild boars, as shown by the low prevalence of infection observed in our study.

In addition to the occasional presence of antibodies in wild boar and barbary sheep, we observed antibodies to *N. caninum* in roe and red deer. Ferroglio and Rossi (2001) reported prevalence of antibodies of 12.7% in red deer and 37.2% in roe deer in the Italian Alps using the *Neospora* agglutination test at 1:40 titer. Similar prevalence of infection was observed in our study in Spanish red deer although notably lower prevalence was observed in roe deer. In addition, in the

present study, prevalence of infection was higher in red deer (11.8%) compared to roe deer (6.1%). Ferroglio and Rossi (2001) also reported prevalence of 29.4% in alpine chamois, while no antibodies in Pyrenean chamois was found in our study.

Although we did not observe presence of *N. caninum* antibodies in Spanish fallow deer, a case of meningoencephalomyelitis in a juvenile animal has been recently described in Switzerland (Soldati et al., 2004).

The absence of *N. caninum* antibodies in 251 wild rabbits indicates that consumption of wild rabbits is not the main route of transmission of *Neospora* to red foxes that have been found to be infected in this area (Almería et al., 2002). The only other serologic analysis of *Neospora* in lagomorphs in Europe showed a low prevalence of infection in hares (8% of 137 *Lepus europaeus*) from East Europe (Ezio and Anna, 2001).

In the present study, the highest prevalence of *Neospora* antibodies was observed in cervids, especially red deer. Antibodies to *N. caninum* in cervids have been observed in black-tailed deer (*Odocoileus hemionus*) (Woods et al., 1994), white-tailed deer (*Odocoileus virginianus*) (Dubey et al., 1999), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) (Ferroglio and Rossi, 2001), moose (*Alces alces*), (Gondim et al., 2004b), pampas-deer (*Ozotoceros bezoarticus*) (Tiemann et al., 2005b), in cervids of genus *Mazama* kept in captivity from Brazil (Tiemann et al., 2005a), in caribou (*Rangifer tarandus*) (Dubey and Thulliez, 2005) and in zoo deer species such as Thorold's deer (*Cervus albirostris*), Eastern elk (*Cervus elaphus canadensis*), Vietnam sika deer (*Cervus nippon pseudaxis*) and Pere David's deer (*Elaphurus davidianus*) (Sedlák and Bártová, 2006). The parasite has been detected through immunohistochemistry in a 2-month-old *Odocoileus hemionus columbianus* found dead in California (Woods et al., 1994) and in a stillborn *Cervus eldi siamensis* at the zoo in Paris, France (Dubey et al., 1996).

In North America, high seroprevalence of *Neospora* in white-tailed deer has been observed in several studies (40.5% of 400, Dubey et al., 1999; 48% of 305 samples, Lindsay et al., 2002; 26% of 193 samples, Gondim et al., 2004b and *N. caninum* has been isolated from this host, Vianna et al., 2005). Additionally, dogs shed *N. caninum* oocysts after being fed with brain of naturally exposed white tailed-deer (Gondim et al., 2004b). All these data are consistent with a sylvatic transmission cycle of *N. caninum* between cervids and canids (Barling et al., 2000; Lindsay et al., 2001; Gondim et al., 2004b; Rosypal and Lindsay, 2005).

Higher prevalence of *N. caninum* antibodies in red deer were observed in fenced estates, that were the majority of estates in the study, and statistically significant differences were observed in *N. caninum* seroprevalence among sampling sites areas (from 0% to 75%). It was interesting to observe that prevalence of infection in red deer was particularly high in some localized areas (MO estate in Guadiana, South-Central Spain or some estates of Catalonia, North-East Spain), while no contact with the parasite was observed in others. In a previous study that analyzed *Neospora* antibodies in wildlife (red deer, roe deer and chamois) from Central (National Park ''Quintos de Mora'') and North-West Spain (Riaño, Mampodre and Ancares hunting estates) no antibodies were observed in the analyzed species by IFAT (González-Zotes et al., 2000). These results suggest that *N. caninum* infection in red deer is not as widespread as in America and seems to be localized to certain areas, where, on the other hand, it can be present in high prevalence. In several of the estates where *N. caninum* was present seroprevalence of infection was even higher than *T. gondii* infection, taking into account that *T. gondii* is a widespread parasite in red deer in Spain (Gauss et al., 2006).

Prevalence of infection of Spanish red deer was not related to age, or sex. The fact that there were not significant differences with age, as has been previously reported (Dubey et al., 1999; Ferroglio and Rossi, 2001), could indicate that vertical transmission was the main route of *Neospora* in this species as occurs with cattle in our area.

Red deer probably become infected by ingesting food or water contaminated by *N. caninum* oocysts excreted by canids in the area. In most of the estates in Spain food is supplied to red deer. In many areas food is left on the ground, while in others it is provided in feeders but in general the place where food supplies are kept is generally open and canids can have access to it. In addition, as in other countries, when red deer are hunted in Spain, carcasses are usually field dressed and the offal is left behind, being available for predators, mainly red foxes, wild and domestic dogs in rural areas. Red foxes are the main wild canid species in Europe where coyotes are not found. Although to date red foxes have not been proved to be a definitive host of the parasite (Schares et al., 2002) some studies have considered the presence of foxes as a risk factor for transmission of *N. caninum* to cattle (Barling et al., 2000; Simpson et al., 1997). In a previous study, prevalence of infection in red foxes studied in Catalonia was 11% (Almería et al., 2002). This prevalence is lower than seroprevalence observed in wolves in North America (39% of 164 wolves), but

similar to 10.6% in 113 coyotes in the same study (Gondim et al., 2004b). These authors explained the differences in *N. caninum* infection in wolves and coyotes as related to their diet. While wolf diet is mainly based on ruminants, coyotes, as red foxes in our area, have a more varied diet (Gondim et al., 2004b). We could observe that red foxes from Vic area (Osona) showed 10% prevalence of infection by PCR in the previous study (Almería et al., 2002) while red deer analyzed in the same area in the present study showed a high seroprevalence of infection (75%). Unfortunately, there were not more areas were both species were analyzed together. The epidemiological role of red foxes in the transmission of *N. caninum* infection would need further investigation.

The present results indicate that in certain areas *Neospora* infection is present in wildlife, with especial importance in red deer and this fact could have important implications in both sylvatic cycles as well as influence the prevalence of infection in cattle farms in those areas. Some control measures such as protection of feedstuff from contamination with canine faeces and a correct disposal of the deer carcasses, that if not most possibly would be available for consumption by a variety of carnivores, should be considered in red deer estates.

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